BASIC MECHANISMS OF HEMOSTASIS*

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THE ponderous literature on the subject of hemostasis could perhaps be considered a classical example of the infinite ability of the human mind for abstract speculation. For several years, the number of working theories of the hemostatic mechanism greatly exceeded and not always respected the confirmed experimental facts. In recent years, however, the revived interest in this field has led to an accumulation of new findings which has been almost too rapid for their orderly incorporation into a logical working pattern. As a result, we have rapidly gone from a state of "orderly ignorance" to one of "confused enlightenment," from which we have not emerged as yet. As new facts and new observations are sorted and critically evaluated, however, they begin to find a proper place and a concept of the hemostatic mechanism is beginning to shape which appears as complex as it is fascinating.

A normal hemostatic mechanism is a very important aspect of human survival. Small, repeated traumas of every day life continuously produce minor injuries to the vessels and, thus, the danger of spontaneous hemorrhage. "Extrinsic" trauma may, by severing vessels, cause bleeding which must be promptly controlled. Both functions require a perfect integration of a number of elementary mechanisms. The vascular wall must present normal resistance and contractility, the platelets and many factors which take part in the coagulation process must be normal in number or concentration, and in activity. Other mechanisms, such as fibrinolysis (which may play a role in limiting the undue extension of the fibrin clot within the vessel) and all the agents which bring about "organization" of the clot, recanalization of the vessel, etc. must also be normal. The very complexity of these mechanisms, then, creates many situations

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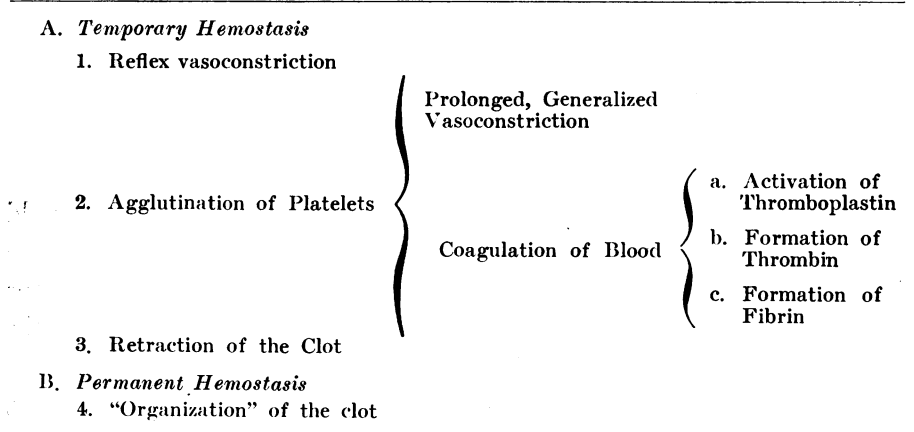
FIGURE 1—PHASES OF THE PROCESS OF HEMOSTASIS IN THE NORMAL MAN FOLLOWING INJURY



This scheme emphasizes the central role of platelets in the process of hemostasis. Platelets: (a) Supply the vasoconstricting agent; (b) Activate thromboplastin, thus initiating the coagulation process; (c) Determine clot retraction.

when hemostasis may become insufficient and bleeding may follow. The intricacies of the hemostatic process can probably be best illustrated by reviewing briefly the sequence of events following injury to a blood vessel. There is, of course, no uniform pattern. Absolute or relative lack of pressure within the lumen of the vessel greatly simplifies the hemostatic mechanism in the case of smaller capillaries and veins. The lumen collapses, the endothelial intimas come in contact and adhere, re-establishing the continuity of the endothelial lining. Thus platelet agglutination and fibrin formation may not even occur in many instances. Bleeding from the arterioles (the most common site of bleeding following "extrinsic" injury) is controlled by a more complex series of mechanisms (Fig. 1). Local, fleeting vasoconstriction caused by an axonic reflex promptly follows the injury to the vessel and the overlying structures. Its physiologic significance is not clear, although, by slowing the circulation, this mechanism may facilitate the accumulation of platelets along the vessel wall and at the site of injury. There platelets promptly agglutinate. In part they act by mechanically plugging the vessel wound. But far more important, however, seems to be their ability to release a series of agents which initiate and govern the following phases of the hemostatic process. The marked, prolonged, more generalized vasoconstriction

which follows platelet agglutination is apparently due to the release of serotonin (5-hydroxytryptamine), either directly or through a plasma-platelet interaction. This is a fundamental step in the hemostatic process since the consequent reduction of blood flow through the vessel wound greatly favors accumulation of thrombin and fibrin formation. Another platelet factor initiates blood coagulation, which is also accelerated by additional platelet constituents. The fibrin clot is finally formed; shortly afterwards it retracts, its size perhaps reduced by fibrinolytic agents. "Organization" of the clot, and recanalization of the vessel follow and very often a new normal endothelium soon lines the vessel in its entirety.

This short description of an hypothesis of the hemostatic process indicates the advisability of individual analysis of the various mechanisms: (a) the vascular mechanism; (b) the platelet factor; (c) the blood coagulation mechanism; (d) the autocatalytic mechanisms; (e) the anticoagulant factors; finally (f) the fibrinolytic mechanism. It should not be forgotten, however, that these various mechanisms are very closely integrated, as is clearly revealed by the study of the pathogenesis of bleeding in various types of hemorrhagic tendency. The single abnormality of one of the hemostatic mechanisms is not necessarily followed by bleeding, if all others are normal. Thus bleeding is moderate and may occur only following serious trauma or operative procedures in pseudohemophilia, congenital deficiency of prothrombin, labile factor, stable factor and fibrinogen, severe as the defect of the hemostatic mechanism involved might be. In many, better defined hemorrhagic syndromes, there is multiple involvement of hemostatic mechanisms. Thus, in some cases of Werlhof's disease, particularly of the acute variety, thrombocytopenia is accompanied by pronounced involvement of the vascular wall (polyarteritis).[1] In parenchymal liver disease, not only is the concentration of the various coagulation factors reduced, but vascular resistance is decreased, while plasma antithrombin titer and fibrinolytic activity are increased. Finally, some of the controlling factors of the hemostatic process may regulate more than one mechanism. A very interesting relationship is that of vitamin K deficiency to vascular fragility.[2,3] In Dicumarol intoxication, hypoprothrombinemia is accompanied by increased vascular fragility (and capillary bleeding is often found). Both abnormalities are promptly corrected by the administration of vitamin K_1. Also in parenchymal liver disease low plasma prothrombin activity and positivity of the tourniquet test are fairly well correlated,

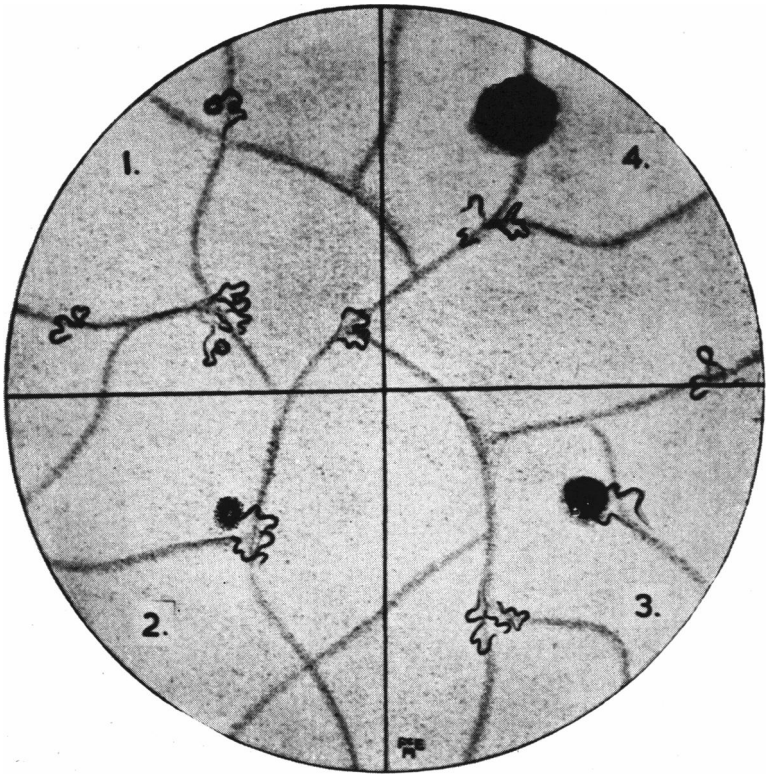


FIGURE 2—STAGES OF FORMATION OF A PETECHIAL HEMORRHAGE IN IDIOPATHIC THROMBOCYTOPENIC PURPURA — There is extravasation of blood (2-3) without apparent lesion of the capillary. Slow reabsorption of extravasated blood (4).

For permission to reprint, see note under Figure 3.

and both may be corrected by the administration of large doses of vitamin K. It may be argued with good reason, therefore, that the subdivision of the hemostatic process into individual mechanisms is probably unwarranted. It is, however, justified if one wants to understand the principal role and significance of each.

THE VASCULAR MECHANISM

The importance of the vascular mechanism of hemostasis is obvious in the control of the response of the vessel to trauma. It is probably equally important in the control of spontaneous bleeding, if one remembers that even marked thrombocytopenia or marked deficiency of blood coagulation factors may not be accompanied by bleeding when, as seen in hypoprothrombinemia, afibrinogenemia, etc., vascular resistance is

not impaired. On the other hand, vascular fragility may cause severe hemorrhagic manifestations although platelets and coagulation mechanism are normal. This situation is best illustrated by the severe bleeding which may follow a small trauma in patients with pseudohemophilia of the vascular variety. In this disease, deficient vascular contractility (prolonged bleeding time) represents the only demonstrable abnormality.

For all its importance, the vascular mechanism of hemostasis is very poorly understood. This is due to many reasons: (a) The evaluation of the status of vascular resistance and of the sufficiency of the response of the vessel to injury is left, at least for practical clinical use, to two very poorly standardized tests: the tourniquet test and the determination of the bleeding time; (b) the mechanism of formation of the elementary bleeding lesions is not entirely clear. Thus, it is thought by many that petechiae represent the result of extracapillary extravasation of red cells and white cells,[4] without demonstrable lesions of the vessel (Fig. 2). Their disappearance in a matter of hours, however, without any discoloration of the overlying skin does not support this contention and there is histologic evidence that petechiae, at least in some stages of their development, may represent aneurysmal dilatations of the capillary wall;[5,6] (c) the site where spontaneous bleeding occurs is also questioned. It may occur from the capillary loop,[5] or, more likely, from the meta-arteriole (terminal arteriole), particularly where collaterals are given out (Fig. 3). The second hypothesis has the support of extremely accurate experimental evidence collected with the direct study of the circulatory changes in the rat meso-appendix and also of data collected by the use of the capillary microscope, which will be described later. The importance of the meta-arteriole in the control of bleeding due to "extrinsic" injury is, on the other hand, obvious since control is achieved in great part by vasoconstriction and capillaries are now known to be devoid of contractile tissue.

There are various causes which may lead to spontaneous bleeding from the meta-arteriole or the capillary. "Spontaneous bleeding" is extravasation of blood from the vessel, due to the inability of the vessel to withstand the pressure within the lumen or to control promptly minor vascular injury inflicted by small traumata such as each of us meets in every day life (contraction of muscles under the skin, articular movements, etc.). The vessels may be *congenitally abnormal*, as presumably they are in hereditary hemorrhagic telangiectasia, vascular pseudohemo-

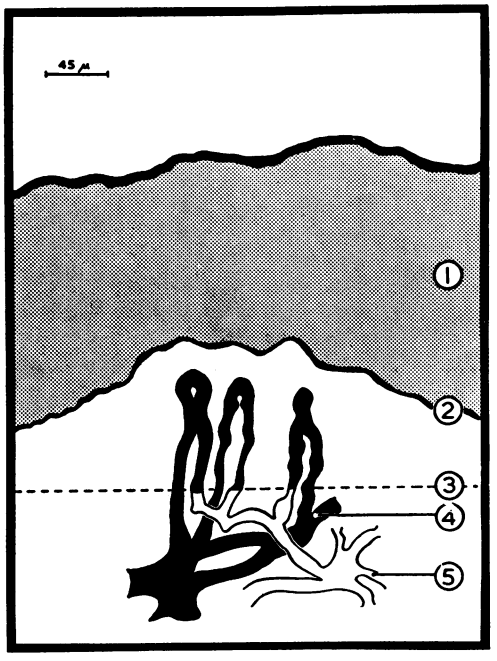


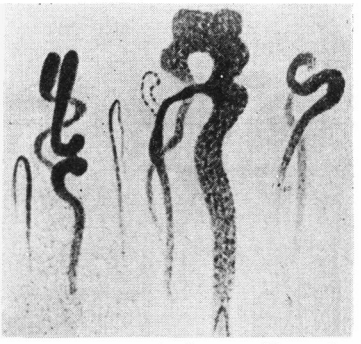
FIGURE 3—SITE OF PETECHIAL FORMATION*: the dotted line (3) indicates where petechiae form. The area in solid black is the one which can be seen under the capillary microscope.

* (1) horny layer of skin; (2) malpighian layers; (4) first collecting venule; (5) terminal arteriole.
Figures 2 and 3 are from J. G. Humble,[4] in *Blood 4:69-75,* 1949. Reprinted by permission of the author and the publishers, Grune & Stratton, Inc.

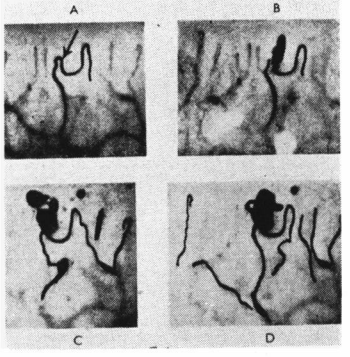
philia, pulmonary hemosiderosis, idiopathic hematemesis and melena, etc. In such instance, bleeding is probably due to primary inability of these vessels to contract following trauma. Or the *permeability* of the vessels may be abnormally high. The capillary wall is composed of fibrillary membrane, a layer of endothelium and a "cementing" substance which unites the individual endothelial cells. Hyaluronic acid is an important constituent of the cementing substance and ascorbic acid is necessary for its synthesis. Spontaneous bleeding follows defect of the "cementing substance," however caused. Experimentally, bleeding follows when one applies hyaluronidase locally to vessels in the rat meso-appendix preparation.[7] Clinically, a defect of the "cementing substance" is found in scurvy. Finally, the *fragility* of the vessels may be higher than normal. This condition makes any minor trauma an important possible cause of severe bleeding.

What changes in the vessels induce increased vascular fragility and spontaneous bleeding is not known at present. Little or no information is obtained with the histologic examination of the vessels, since, with the already mentioned exception of some cases of acute idiopathic thrombocytopenic purpura, no significant histologic lesion is detectable in most cases with hemorrhagic tendency. Fragmentary information has been obtained with studies "in vivo." As mentioned already, Humble[4] witnessed petechial formation in patients with hemorrhagic tendency with or without thrombocytopenia by the elegant technique of following changes in the visible capillaries of the nail bed under the experimental conditions of the tourniquet test. In all cases, hemorrhage occurred from the arterial end of the capillary loop (Fig. 3), where, as Landis[8] has demonstrated, the pressure within the capillary is the highest and the vessel suddenly dilates into the capillary loop. There was no demonstrable obliteration of the vessels during or after hemorrhage. Thus, inability to contract was of paramount importance in explaining the increased vascular fragility and the spontaneous bleeding when the intravascular pressure was elevated. Humble's findings on the mechanism of bleeding in hemorrhagic disease are very similar to those obtained by MacFarlane[9] with regard to the response to trauma of the capillary loop in various hemorrhagic diseases (Fig. 4). Methods such as used by Humble and MacFarlane are not without criticism. Thus, in MacFarlane's work, disappearance of the capillary loop was considered evidence of vasoconstriction. In fact, it may only have represented "skimming" or emptying of the capillary loop since the method visualized the contents and not the walls of the capillary. Figure 4/2 shows, to the left, a normal nail-bed field: some capillary loops are clearly defined, others less so, possibly due to "plasma skimming"; to the right, is a picture of what happens when the loop is damaged (by quartz needle puncture, in this case). The injured vessel disappeared from view (it did not reappear again for as long as one-half to two hours). At the height of vasoconstriction, this was so intense that the arterial end of the capillary loop could withstand internal pressures as high as 100 mm. Hg. It is not difficult to understand how this prolonged, intense vasoconstriction must have favored the clotting of blood within the loop. Figure 4/3 shows studies in patients with primary vascular defect (pseudohemophilia) as well as thrombocytopenia. The capillary loops often appeared distorted. In both instances little or no vascular contraction took place and bleeding con-

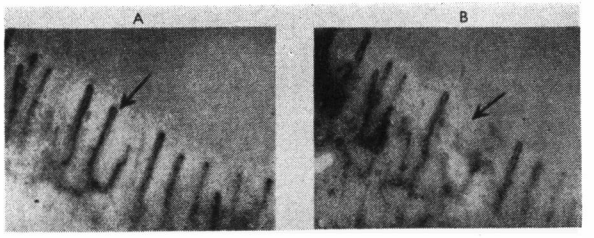
FIGURE 4—RESPONSE OF HUMAN CAPILLARIES TO INJURY.



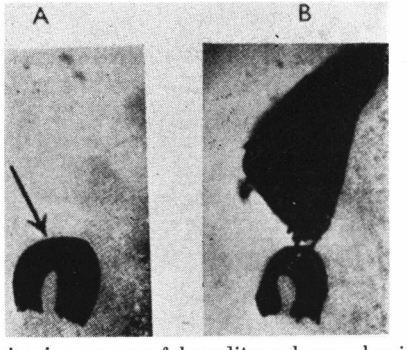
(1) Changing appearance of the capillary loops in normal human fingernail bed.
(After Parrisius; Deutsche Zeitschr F. Nervenheilk., 1921)



(3) Capillaries in a case of vascular purpura.
A—Capillary loops appear irregular and distorted.
B, C, D—No contraction of the loop immediately, 2′ and 5′ after injury blood is accumulating around the vessel.



(2) Normal capillary loops in human nailbed.
A—Normal capillaries.
B—Injured capillary loop has disappeared (contracted?).



(4) Capillaries in a case of hereditary hemorrhagic telangiectasia.
A—Greatly distended capillary.
B—No contraction of the loop after injury. Large hemorrhage.

Fig. 2, 3, 4 from MacFarlane,[9] Quart. J. Med., 1941. Reprinted by permission.

tinued for minutes, until finally arrested by outside pressure of the extravasating blood. A similar picture was found in congenital abnormalities of the vascular system, such as hereditary hemorrhagic telangiectasia (Fig. 4/4). In this condition also the capillary loop was greatly enlarged and distorted. Lesion by the needle was followed by large hemorrhage. Studies like those by Humble and MacFarlane, which have been repeated and confirmed by others, have been very fruitful in supplying information as to the role of vessels in hemorrhage and hemostasis.

Lack of vasoconstriction following trauma and poor contractility of the vessel may then be one of the important causes of spontaneous bleeding or of defective hemostasis after trauma. The pathogenesis of both abnormalities is not clear, with the exception, perhaps, of the thrombocytopenic states. The brilliant experimental work by M. B. Zucker,[10] confirmed in man by H. D. Zucker[11] by the painstaking method of studying histologically serial skin sections of thrombocytopenic patients, has done much to illustrate the role of platelets in vasoconstriction and vasocontractility. When, however, no platelet deficiency can be demonstrated, the vessel's permeability may be grossly abnormal or other factors controlling vasoconstriction may be deficient. This brings us to a short discussion of some other factors which may influence the response of the vessel to trauma or control spontaneous bleeding. Knowledge in this field is sorely inadequate.

As mentioned already, ascorbic acid is necessary for the synthesis of the cementing substances of the capillary. The vitamin has been used with variable success in the treatment of ill-defined hemorrhagic syndromes due to increased vascular fragility (or permeability?), and to antagonize the bleeding due to Dicumarol overdosage. Favonoids of various types will (perhaps aspecifically) improve the bleeding tendency of that heterogeneous group called "vascular purpura" if given at high doses and over a long period of time. The endocrine system also plays a role in the control of the vascular mechanism of hemostasis. Ungar[12] has isolated from the spleen a substance ("splenin") which shortens the bleeding time, increases the vascular resistance and inhibits the release of histamine from injured cells. Liberation of "splenin" is mediated through the pituitary gland. Close in properties to splenin is "thrombocytosin"[13] a steroid, which, in addition to an effect on the number and function of circulating platelets, also improves the vascular resistance and is said to produce remission of bleeding in patients with idiopathic

FIGURE 5—SPECIFIC PLATELET CONSTITUENTS WITH BIOLOGICAL ACTIVITY

1. Vasoconstrictor Principle (Serotonin = 5 Hydroxytryptamine)
2. Clot Retracting Agent (?) = Retractoenzyme
*3. Platelet Thromboplastic Factor
   +Plasma Factors { Antihemophilic Globulin, PTC, PTA (?) } ⟶ Thromboplastin
4. Platelet Factor 1 (Affects conversion of prothrombin to thrombin)
5. Platelet Factor 2. (Affects conversion of fibrinogen to fibrin)
*6. Platelet Factor 3 (Antiheparin)
7. Antifibrinolysin (?)
8. Histamine
9. Hypotensive Principle

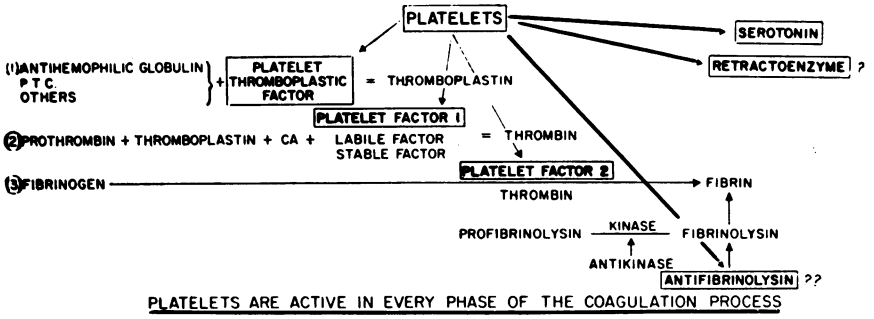
* Probably identical.

thrombocytopenic purpura even without elevation of the platelet count. Finally, ACTH and cortisone, while having only a variable effect on the platelet count and occasionally accelerating the clotting time, cause prompt and striking improvement of capillary resistance[14] and, rarely, a reduction of the bleeding time after prolonged administration. The mechanism of this effect is not clear but these hormones are used very successfully to control the clinical bleeding of patients with thrombocytopenia, megakaryocytic or amegakaryocytic (in addition to platelet transfusions), vascular pseudohemophilia and undefined "vascular purpura." The striking clinical response to ACTH of thrombocytopenic patients even in the absence of any effect on the platelet level, is in itself additional evidence of the great importance of the vascular factor in the control of hemostasis.

THE PLATELET MECHANISM

Bizzozero[15] was perhaps the first investigator to realize fully the unique importance of platelets in the physiologic process of hemostasis and in the pathogenesis of thromboembolism. Recent studies have indeed emphasized the conclusion that platelets exercise a very complex and fundamental effect on the hemostatic process and the coagulation of blood in particular (Fig. 5). There is little doubt that the control of bleeding following injury to the vessels is greatly dependent on the function of platelets. As viscous bodies they mechanically plug the vessel wound. They cause vasoconstriction and initiate blood coagulation

FIGURE 6—THE FUNCTION OF PLATELETS IN THE HEMOSTATIC PROCESS (SCHEMATIC).

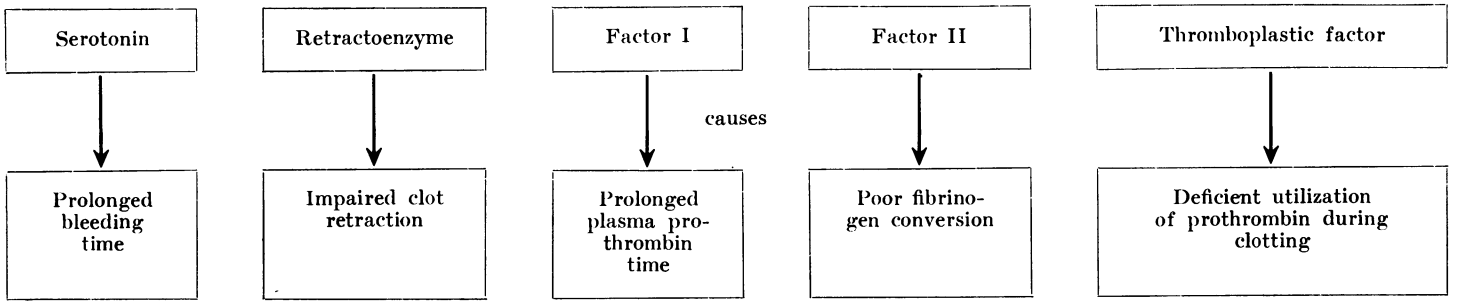


through the release of chemical agents at the time they agglutinate and disintegrate at the site of the vascular injury. It has been thus far believed that only intact, normally functioning platelets are able to determine clot retraction. More recently, however, Fonio[16] has suggested that clot retraction is due to a specific constituent of platelets (retractoenzyme), which has been isolated from the hyalomere of platelets. These results are stimulating, but need confirmation. Platelets are veritably small physiologic units, of extremely complex enzymatic and chemical structure. In addition to their function in the hemostatic process, platelets liberate a hypotensive principle and contain antifibrinolysin. As previously pointed out, platelets regulate practically all phases of the hemostatic process: (a) The generalized vasoconstriction which follows vascular injury is due, at least in part, to the release of serotonin; (b) disintegration of platelets releases the thromboplastic factor, which reacts with plasmatic agents to form thromboplastin, and factors 1 and 2 which accelerate conversion of prothrombin to thrombin and formation of fibrin respectively;[17] (c) platelets oppose the activity of heparin, probably by releasing another constituent (factor 3) which is probably identical with the platelet thromboplastic factor (Fig. 6).

The complex function of platelets is also demonstrated by the severity of the hemostatic defect in thrombocytopenic states. Thrombocytopenic patients show prolonged bleeding time and positive tourniquet test (increased vascular fragility), reduced utilization of prothrombin during clotting and increased sensitivity to heparin (deficiency of thromboplastic factor and/or factor 3); moderately delayed one-stage plasma prothrombin time (deficiency of factors 1 and 2), absence of clot retrac-

FIGURE 7—THROMBOCYTOASTHENIAS

Hemorrhagic diatheses due to deficiencies of one or more of the platelet factors



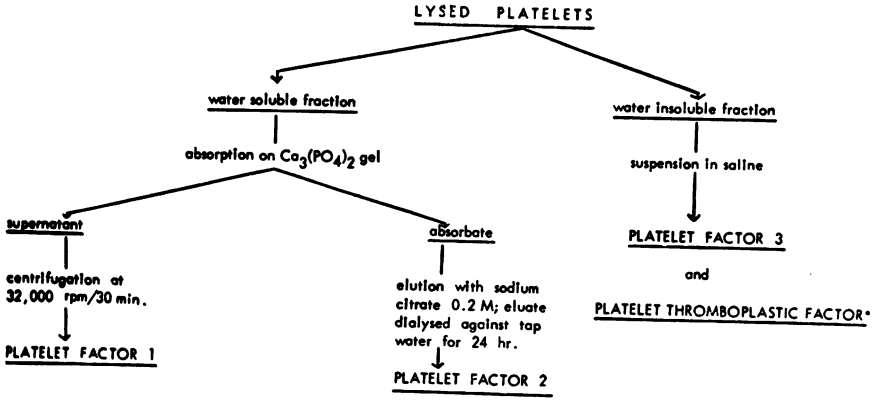
A GROUP OF RECENTLY STUDIED CASES

Name	*Age*	*Sex*	*Platelet count (x 10³/cu. mm.)*	*Bleeding time (Ivy) minutes*	*Prothrombin Activity Plasma %*	*Prothrombin Activity Serum %*	*Clot retraction %*
E.K.	16	M	567.0	*35*	95	*90*	31
R.D.	6	F	436.8	3	90	17	*2*
G.J.	47	F	549.1	6½	85	*92*	34
R.M.	4	F	501.0	*22*	80	15	39
E.K.	29	F	497.3	4	*30*	6	32

Selective, combined, total dysfunctions of platelet functions may occur. Common features included: (a) bleeding, common after injury or at surgery, spontaneous hemorrhage less common; (b) presence of large and bizarre platelets; (c) correction of the hemostatic defect by the transfusion of platelets.

Of the above group, the hemostatic defect of E.K. was one of the thromboplastic factor and of a hypothetical "vascular factor"; that of case R.D. was due to single deficiency of "retractoenzyme" (a hypothetical factor controlling the clot retraction); that of G.J. was due to single deficiency of platelet thromboplastic factor; that of R.M. was due to deficiency of "vascular factor" alone; that of E.K. to deficiency of platelet factors I and II.

FIGURE 8*—CRUDE SEPARATION OF THE PLATELET FACTORS TAKING PART IN THE PROCESS OF BLOOD COAGULATION



* No practical method of separation of the two water-insoluble factors is available at present. Figures 8, 9, 12 and 13 are from Stefanini, M.[22] in *Amer. J. Med. 14*:64-86, 1953. Reprinted by permission.

tion. The relationship of individual platelet factors to specific defects of the hemostatic mechanism, on the other hand, is illustrated by the findings in cases of thrombocytoasthenia.[18] In this hemorrhagic tendency, platelets are normal in number but they exhibit morphologic abnormalities (abnormal size, appearance and staining) and failure of one or more physiologic functions (Fig. 7). Clot retraction, utilization of prothrombin during clotting, slightly prolonged one-stage plasma prothrombin time, positive tourniquet test are present as isolated findings or in various combinations in these patients. In all, the hemostatic defect is corrected by the administration of viable platelets. Recently, platelets have become available in large amounts.[19,20] It has thus become possible to fractionate them and obtain individual chemical constituents in relatively purified form (Fig. 8). This approach has confirmed the existence of platelet factors with specific functions. As a result of these studies, some of the chemical constituents of platelets have been fairly well characterized by Van Creveld and Paulssen[21] and in our Laboratory (Fig. 9).[22]

However, while very detailed information has been obtained with regard to the physiologic structure and the function of platelets in hemostasis, we still lack very important and basic information. We cannot, at the present time, visualize clearly the mechanism which causes ag-

FIGURE 9—CHARACTERIZATION OF SOME OF THE PLATELET FACTORS

Platelet factor 1:*

(1) accelerates the conversion of prothrombin to thrombin
(2) water-soluble; precipitated from solution by 50% saturation with $(NH_4)_2SO_4$
(3) sediments following centrifugation at 32,000 r.p.m. for 30 min.
(4) heat labile (53°C.)
(5) non-absorbed on $Ca_3(PO_4)_2$ gel or $BaSO_4$

Platelet factor 2:

(1) accelerates the

$$\text{fibrinogen} \xrightarrow{\text{thrombin}} \text{fibrin reaction}$$

(2) water-soluble; does not precipitate from solution following centrifugation at 32,000 r.p.m. for 30 min.
(3) heat stable
(4) absorbed on $Ca_3(PO_4)_2$ gel and $BaSO_4$; can be eluted from them with sodium citrate

Platelet factor 3:†

(1) opposes the activity of heparin in the blood coagulation process
(2) water-insoluble, suspendable in saline solution
(3) relatively heat stable
(4) non-absorbed on $Ca_3(PO_4)_2$ gel or $BaSO_4$

Platelet thromboplastic factor:†

(1) interreacts with one or more plasma components to form active thromboplastin
(2) found mostly in platelets but also in other formed elements of the blood
(3) water-insoluble, partly soluble in citrate-phosphate buffer solution
(4) heat stable (relatively, at 56° C)
(5) precipitates following centrifugation at 32,000 r.p.m. for 30 min.
(6) high phospholipid content: similar in chemical structure to placental thromboplastin

* Many of the properties of this factor are shared by the "serum accelerator." The relationship of the two agents is a very close one.

† Platelet factor 3 and platelet thromboplastic factor are very similar in properties and are probably identical agents.

glutination of platelets at the site of vascular injury and we cannot explain why platelets are so unstable in contact with foreign surfaces. Equally little is known concerning the mechanism of platelet production from the megakaryocytes and of their delivery from the bone marrow into the peripheral circulation. We have only fragmentary knowledge of the factors which regulate the platelet level in the peripheral blood (possibly the same ones which regulate platelet production and delivery). Nutritional deficiencies (scurvy, pernicious anemia, etc.) cause reduced platelet formation; "hypersplenism" inhibits delivery and reduces the production of platelets by the megakaryocytes. The platelet level may be temporarily elevated by the administration of high doses of ACTH

FIGURE 10—WHERE THE VARIOUS COAGULATION FACTORS ARE FOUND

Platelets	*Plasma*	*Serum*
1. Platelet thromboplastic factor (platelet activator)	(a) *Agents favoring blood coagulation*	
2. Platelet factor 1	1. Antihemophilic globulin (AHG)	
3. Platelet factor 2	2. Plasma thromboplastin component (PTC)	1. PTC
4. Platelet factor 3 (anti-heparin factor)	3. Plasma thromboplastic antecedent (PTA)	2. PTA (?)
	4. Prothrombin	
	5. Calcium	
	6. Labile factor →	3. "Serum accelerator"
	7. Stable factor →	4. "Activated" (?) stable factor
	8. Fibrinogen	
	(b) *Agents opposing blood clotting or destroying the formed clot*	5. Thrombin 6. Metathrombin
	9. Antithromboplastins	(*)
	10. Antithrombins	
	11. Albumin X	
	12. Profibrinolysin	
	13. Antifibrinolysin	
	14. Antifibrinolysokinase	

* It also contains factors 9 to 14 of plasma, in relatively unmodified concentration.

FIGURE 11—CHARACTERISTICS AND PROPERTIES OF THE PLASMATIC

	Abbreviation	*Function in the Clotting Process*	*Its Deficiency Causes*	*Name of Clinical Bleeding Disease*	*Found in Cohn's Fract.*	*Precipitated from Plasma by:*	*Fate During Clotting of Blood*
Anti-hemophilic Globulin	AHG	+ Platelets, PTC, (?) PTA Forms Thromboplastin	Deficient Utilization of Prothrombin During Clotting	Hemophilia	I	(a) Saturation with CO_2 of Diluted Plasma (b) 33% Saturation with $(NH_4)_2SO_4$	Quickly and Almost Completely Utilized
Plasma Thrombo-plastic Component	PTC	+ Platelets, AHG, (?) PTA Forms Thromboplastin	—Ditto—	PTC Deficiency	IV	40-50% Saturation with $(NH_4)_2SO_4$	Almost Completely *non* Utilized
Plasma Thrombo-plastin	PT	+ Calcium, Labile and Stable Factor Converts Prothrombin to Thrombin	—Ditto—			25% Saturation with $(NH_4)_2SO_4$	Utilized in Great Part
Pro-thrombin		+ Calcium, Labile and Stable Factor, Thromboplastin Produces Thrombin	Poor Formation of Thrombin	True Hypo-prothrom-binemia	III-2		75-85% Utilized for Formation of Thrombin
Stable Factor	SF	+ Calcium, Labile Factor, Thromboplastin, Prothrombin Produces Thrombin	Poor and Slow Formation of Thrombin	False Hypo-Prothrom-binemia (Convertin Deficiency)	III-2		Very Little Utilized
Labile Factor	LF	+ Calcium, Stable Factor, Thromboplastin, and Prothrombin Produces Thrombin	—Ditto—	False Hypo-prothrom-binemia (Parahemo-philia)	III	(a) 10% Cold Ether, pH 5.4 from Dil. Pl. (b) 45% Saturation with $(NH_4)_2SO_4$	Utilized and Activated to Serum Accelerator
Fibrinogen		Converted to Fibrin by Thrombin	No or Poor Blood Clot	Hypo- and Afibrino-genemias	I-2	(a) Weak Acids (b) 8% Cold Ethanol (c) 50% Saturation with NaCl; 25% Saturation with $(NH_4)_2SO_4$	Completely Converted to Fibrin
Thrombin		Converts Fibrinogen to Fibrin			Albumin	Acidification to pH 5.1 in the Cold	See: Mechanisms of Thrombin Inactivation
Profibrin-olysin		Lysis of Clot after Activation only	Activation causes excessive blood destruction. Deficiency may cause thrombotic tendency	Purpura Fibrinolytica	III-3	(a) 33% Saturation with $(NH_4)_2SO_4$ (b) Acidification to pH 5.3	

ACTORS OF BLOOD COAGULATION AND THEIR DERIVATIVES

Fate During Storage of Plasma	*Heat Stability*	*Solubility*	*pH Range of Activity*	*Effect of Dicumerol on Conc.*	*Absorption on Gels and Filters*	*Remarks*
50% Destroyed in 12 Hours, table in Frozen, yophilized Pl.	Stable at 56°C	Water Soluble		None	None	
Stable	Stable at 56°C for 30′		4-11.2	None	Absorbed from Oxalated Plasma, Can be Eluted with Sodium Citrate	
Relatively Stable (50% Loss in 1 Week)	—Ditto—	Water Soluble	Wide	None	Absorbed from Citrated Plasma, Can be Eluted with Saline	
Stable	Relatively Labile (40-60°C)	Water Soluble	Isoelectric Point 4.2	Concentration Reduced	Absorbed from Oxalated Plasma, Can be Eluted with Sodium Citrate	α-Globulin, S-containing Glycoprotein
Very Stable	56°C for 30′		4-8	—Ditto—	—Ditto—	Retained on 30% Asbestos Filter (Cow Plasma)
Very Labile (More Stable in Citrated Plasma)	Destroyed at 56°C	Water Soluble	4-10.5	None	None	Restores Clotting Defect of Aged Plasma
Fairly Stable	Clots Irreversibly at 56°C	Soluble in Saline Solution	Isoelectric Point 5.3	None	None	*Fibrin* Has Essentially Similar Properties
	Relatively Labile (40-60°C)	Water Soluble		Indirectly Decreased Concentration	None	Contains S and Carbohydrates
	Relatively Labile	Water Soluble	4.5-8	None	None	

and cortisone and, at times, by the administration of extracts of the thymus gland,[23] of polycythemic blood[24] and blood from recently bled donors. These may all contain megakaryocyte-stimulating factors. Transfusion of normal plasma, on the other hand, causes in many cases temporary reduction in the platelet count, due to the activity of a thrombocytopenic globulin.[25] Even without commenting on changes in viscosity, adhesiveness and fragility of platelets, which have been repeatedly discussed in relation to "blood hypercoagulability" and thromboembolic tendency, it is easy to see that many questions remain unanswered with reference to the role of platelets in hemostasis.

The Blood Coagulation Mechanism

In addition to those supplied by the platelets, a number of plasma factors take part in the process of blood coagulation (Fig. 10). They can be divided into three groups: (a) those needed for the formation of thromboplastin: antihemophilic globulin (AHG), plasma thromboplastic component (PTC), ? plasma thromboplastic antecedent (PTA); (b) those entering into the formation of thrombin: prothrombin, calcium, labile factor, stable factor; and (c) fibrinogen. The characteristics and properties of the various agents are presented in Figure 11. Several denominations have been recommended for many of these factors. Synonyms for the various clotting agents are presented in Figure 12. There are, in addition, agents which oppose the formation of the fibrin clot: natural antithromboplastin and antithrombins, albumin X; and factors which are part of the fibrinolytic mechanism: profibrinolysin, antifibrinolysokinase, antifibrinolysin.

The fate of these factors during the clotting process is very significant in understanding their role in blood coagulation. Many are utilized in part or completely, others are not and are found in unchanged concentration or activity in the serum. The first group probably enters in quantitative interactions during the coagulation of blood; those of the second group most likely act as catalysts of the reaction. Fibrinogen is completely utilized; very little AHG and prothrombin are left in the serum after completion of clotting. Labile factor is almost completely utilized, being converted to the more active agent, the serum accelerator. This agent, in turn, disappears quickly after completion of coagulation. PTC and stable component, and calcium are little or not at all utilized during clotting and their concentration is practically unchanged.

FIGURE 12—SYNONYMS OF VARIOUS FACTORS IN THE COAGULATION OF HUMAN BLOOD(*)

1. *Factors taking part in the activation of thromboplastin:*

PLATELET THROMBOPLASTIC FACTOR
- thromboplastinogenase
- platelet activator
- cellular thromboplastic component (TCC)†

PLASMA THROMBOPLASTIC FACTOR
- prothrombokinase
- plasmakinin
- antihemophilic globulin
- thromboplastinogen
- thrombocytolysin
- thrombokatalysin
- thromboplastic plasma component (TPC)†

2. *Tissue thromboplastin*
- thrombokinase
- cytozime
- thromboplastic protein
- thrombokinin

† Not to be confused with PTC (plasma thromboplastic component) (see text)

3. *Factors involved in the conversion of prothrombin to thrombin* (other than calcium and thromboplastin)

"LABILE FACTOR"
- thrombogène
- component A of prothrombin
- factor V → factor VI
- accelerator factor
- co-factor of thromboplastin
- plasma Ac-globulin → serum Ac-globulin
- proprothrombinase → prothrombinase
- prothrombinogenase → thrombinogenase
- ? prothrombinokinase → thrombokinase(**)
- plasma prothrombin conversion factor (PPCF) → serum accelerator
- proaccelerin → accelerin

"STABLE FACTOR"
- co-factor V
- ? component B of prothrombin
- prothrombin accelerator
- prothrombin conversion factor
- prothrombin converting factor
- co-thromboplastin
- plasma precursor → serum prothrombin conversion accelerator (SPCA)
- proconvertin → convertin
- factor VII
- ? prothrombinogen (inactive prothrombin)
- kappa factor (in chicken)

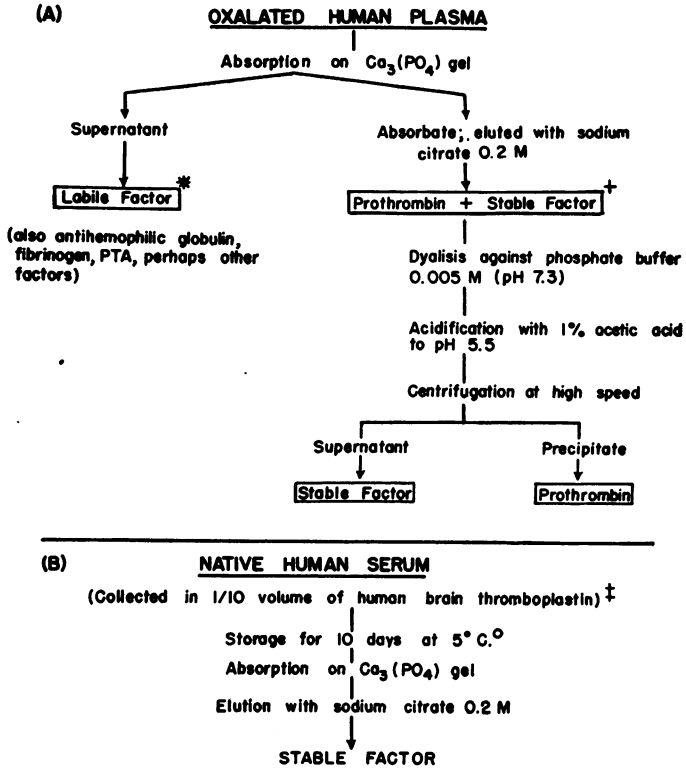
(**) In the meaning of an agent capable of influencing the conversion of prothrombin to thrombin

* Many of the factors in group 3 are very likely: (a) mixtures of "labile factor" and "stable factor"; (b) even more probably, mixtures of "stable factor" and "prothrombin"; (c) possibly, intermediate steps in the prothrombin → thrombin conversion and thus prothrombin derivatives.

Some of the factors are arranged in couples, joined by an arrow. Those at the right of the arrow are considered less active precursors found in plasma; those to the left of the arrow, the active (or more active) form found in the serum. For this reason, some investigators prefer to think of "labile factor" and "stable factor" in terms of a system and not as an isolated agent.

Characterization of most of the known coagulation factors has now proceeded to a point where their existence can no longer be doubted. Perhaps some doubt remains with regard to the relationship of prothrombin to the stable factor, since the physical and chemical characteristics of the two agents are practically identical. Prothrombin and stable factor can be separated, at considerable loss of activity of both agents, by

FIGURE 13—CRUDE SEPARATION OF THE PLASMA FACTORS TAKING PART IN THE CONVERSION OF PROTHROMBIN TO THROMBIN.



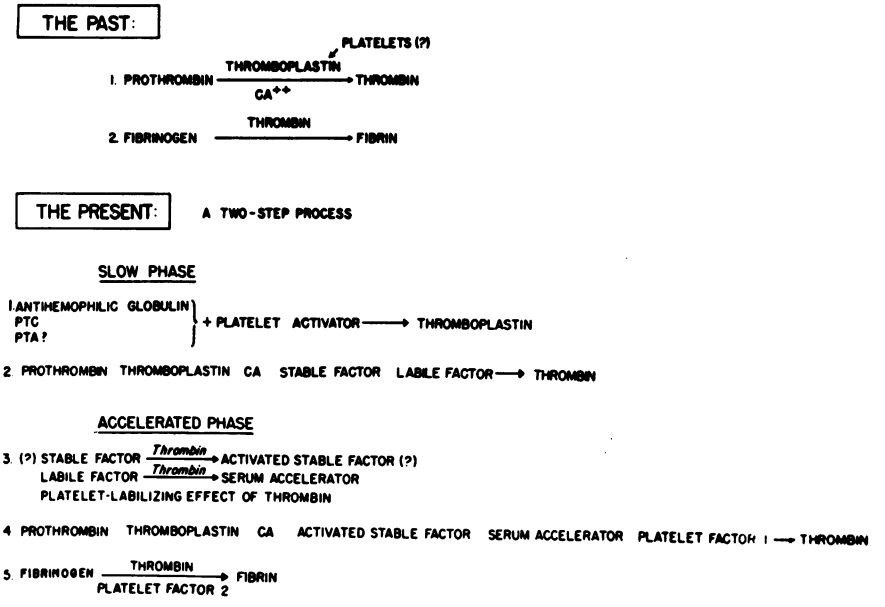
* The "labile factor" can be specifically precipitated by acidifying the diluted plasma to pH 5.4.
\+ In bovine plasma prothrombin and "stable factor" can be separated easily (although with large loss of both factors) by filtering the plasma through 30% asbestos filters. The "stable factor" is retained on the filter, prothrombin passes through. The "stable factor" can then be eluted with sodium citrate from the filter pad.
‡ To cause complete utilization of prothrombin, thus eliminating this agent from the serum.
° To destroy or inactivate the "labile factor" not utilized during the process of blood coagulation and the "serum accelerator" developed during the clotting of blood.

absorption on asbestos filters (cow plasma),[26] by chromatographic techniques (human plasma),[27] and by chemical separation.[28] But the possibility remains that stable factor might represent either a less active precursor[29] or a derivative of prothrombin. Many of the factors can be obtained from plasma in a highly purified state, but only at the cost of great loss of all others. Figure 13 illustrates the separation of prothrombin from stable and labile factor. Final products from plasma and serum are of relative purity only.

The study of the interaction of the various individual factors represents the most difficult part in the study of the blood coagulation mechan-

FIGURE 14

THEORIES OF THE MECHANISM OF BLOOD COAGULATION



ism. The theories of various investigators in the field have been reviewed recently [22] and they will not be discussed here. On the basis of careful analytical study and of the results of tests indicating with sufficient accuracy the nature of the hemostatic defect in the bleeding patient, it is now generally felt that the process of blood coagulation can be divided into at least three phases (Fig. 14). Many others must certainly exist and are being gradually recognized. The first phase is the *formation of thromboplastin.* The bleeding diseases of this group (hypothromboplastinemias) have in common a deficient utilization of prothrombin during clotting and include the hemophilia syndrome and the thrombocytopenic states. At least three and possibly four factors take part in the reaction: platelet activator or thromboplastic factor, AHG, PTC and, possibly, PTA. This phase of the clotting process has probably received the greatest amount of attention in the past year. After the discovery by Quick[30] and by Brinkhous[31] that the interaction of platelet and plasmatic factors was needed to form thromboplastin, one advance quickly

followed another. Hemophilia was recognized to be a disease of defective formation of thromboplastin. The exhaustive study of this disease, however, and particularly of the clotting ability of hemophilic blood and of the therapeutic response of hemophiliacs to various agents revealed a number of paradoxes: 1) the ability of some hemophilic-plasmas to correct each other's defect; 2) the possibility of obtaining normal yield of AHG from plasma of some hemophiliacs; 3) the lack of response of some hemophilic patients to the administration of purified AHG; 4) the favorable response to *stored* plasma of some cases of hemophilia, but not of others (requiring, on the contrary, *fresh* blood). The interpretation of these observations on which, I am sure, Dr. Brinkhous will comment more fully later, has come from the work of three main groups of investigators: Schulman and Smith,[32] Aggeler and co-workers,[33] and Biggs and co-workers[34] in England. It has led to the discovery of PTC. A further extension of this work has come from Rosenthal and co-workers[35] which has suggested that another factor (PTA) may take part in the formation of thromboplastin. PTC is strikingly different from AHG and, if not a derivative of the platelet-AHG interaction, should represent a new clotting agent. More doubt has been expressed with regard to PTA. The deficiency of this factor has been suggested by some[36] to represent a combined deficiency of PTC and AHG. The properties of PTA are, indeed, intermediate between those of PTC and AHG. If this is true, it would avoid the postulation of a new clotting agent to explain the results of Rosenthal et al.[35] A more complete study of this agent would certainly be welcome and, I am sure, is under way by its discoverers.

The nature of the reaction among the three and, possibly, four factors is not completely clear. AHG and platelet thromboplastic factor interact according to definite quantitative proportions[37] and are both utilized almost completely during the coagulation of blood. The role of PTC, however, is not clear as yet, since the factor is found in practically unmodified concentration in the serum and may act only as a catalyst of the AHG-platelet reaction. In any case, studies by MacFarlane and co-workers ("thrombin generation test"), by Biggs and co-workers ("thromboplastin generation test"), have done much to prove that deficiency of AHG (hemophilia), PTC (PTC deficiency) and PTA (PTA deficiency) belong together in the group of diseases where the activation of thromboplastin is limited and slow. We have reached the same con-

clusion by the study of the concentration of plasma thromboplastin in these patients. Dr. Campbell, now of Portland, Ore., and I have been able to isolate from citrated plasma a thromboplastic agent which develops when platelet-rich human plasma is incubated in glass. A method for the direct determination of the activity of plasma thromboplastin[38] has been developed showing that this intermediary factor is only slowly and incompletely formed in patients with hemophilia, PTC or PTA deficiency, and thrombocytopenia and thrombocytoasthenia of some type. It may be added that the successful isolation of plasma thromboplastin is one of the first attempts to separate from human plasma products which represent not primary factors, but rather intermediary phases of the blood coagulation mechanism.

The nature of the second phase of the coagulation process, the *conversion of prothrombin to thrombin*, is the source of bitter controversy. Abnormalities of this phase include the hypoprothrombinemias, characterized by a delayed one-stage prothrombin time of plasma. Thromboplastin (formed during the first phase of the coagulation process), calcium, two accessory factors (labile and stable) are necessary to convert prothrombin to thrombin. By some investigators this conversion is interpreted as a stoichiometric process. Others, however, consider thromboplastin, calcium, labile and stable factors as mere accelerators of the conversion of prothrombin to thrombin. Facts, often obtained with a completely different experimental approach, can be quoted in support of one or the other point of view. Thus (a) thrombin does not contain thromboplastic material or ionic calcium (but may contain bound calcium); (b) the molecular weight of thrombin is smaller than that of prothrombin; (c) thrombin evolves slowly but spontaneously in a solution of purified prothrombin in 25 per cent sodium citrate;[39] (d) the stable factor is apparently not utilized during the process of blood coagulation.[40] If one accepts these results, one must conclude that prothrombin contains all materials necessary for the formation of thrombin and that calcium, thromboplastin, stable and labile factors can affect only the rate of the conversion of prothrombin. On the other hand: 1) concentration of available thromboplastin and of calcium is the limiting factor in the amount of prothrombin which can be converted to thrombin; 2) labile factor is utilized quantitatively in the formation of thrombin, serum accelerator evolving at the same time;[41,42] 3) no thrombin can apparently evolve in a mixture of prothrombin, thromboplastin and

calcium in the absence of labile factor.[43] The same holds true, in our experience, in the case of the stable factor. These findings would then suggest that prothrombin, calcium, thromboplastin and, at least, labile factor interact according to definite quantitative proportions, and that the reaction may be stoichiometric in nature. All these experiments, which are perhaps too hastily translated into established facts, are, however, not above criticism. To comment only about two of them, on which depends heavily the thinking of the opposing groups of investigators, one would have reason to dispute the "purity" of many of the isolated coagulation factors used in these experiments. Despite remarkable efforts on the part of many, absolutely pure coagulation factors are not available as yet. On the other hand, decrease in concentration or activity of any coagulation factor during the formation of thrombin is not definite evidence of a stoichiometric reaction, especially when it is known that, while some of these factors decrease, more active ones develop.

There are other findings further confusing the kinetics of the second phase of the coagulation process. Evidence that calcium may act in the coagulation of blood only when bound to proteins has been reviewed and strengthened.[44] The relationship of prothrombin to thrombin remains partially unsolved. The two agents present certain affinities of chemical composition, but the molecular weight of thrombin is smaller than that of prothrombin. Recently Lorand et al.[45] have studied the mechanism of formation of thrombin from prothrombin in 25 per cent sodium citrate solution. Thrombin formation, in their experiments, proceeded in two steps. Carbohydrate and nitrogen were released first from the prothrombin molecule; thrombin was then formed autocatalytically from the intermediate derivative of prothrombin. These experiments support the hypothesis that thrombin is a product of "degradation" of prothrombin, but do not help greatly in understanding the role of the "accessory" factors (thromboplastin, calcium, labile and stable factors) in the formation of thrombin besides implying that they are mere accelerators.

Flynn and Coon[46] have recently presented a veritable contribution to the understanding of the second phase of the coagulation mechanism. It has been thought for some time that several reactions take place during the conversion of prothrombin to thrombin, some of them involving preliminary interaction of thromboplastin, labile and stable factors, and calcium to form intermediate complexes. Thus, Owren[26] has postulated

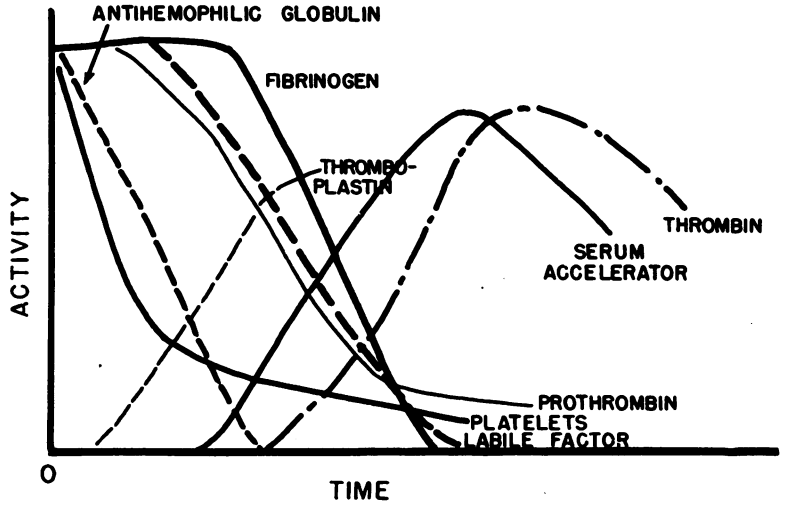
that, in an early phase of the process of thrombin formation, thromboplastin and calcium react with proconvertin to form convertin. Flynn and Coon have demonstrated by direct techniques of isolation that thromboplastin and labile factor, thromboplastin, calcium and stable factor are able to form intermediate complexes, none of which, however, can convert prothrombin to thrombin. The mixture of thromboplastin, calcium, labile factor and stable factor, however, forms a complex (quickly settling in the centrifuge), which easily converts prothrombin to thrombin. These observations not only reemphasize the step-like nature of the second phase of the coagulation mechanism, they also support the contention that many of the accelerators of the conversion of prothrombin to thrombin described in the literature may, in effect, be intermediary complexes of two or more of the factors taking part in the reaction. It may probably be concluded on the basis of the available evidence that the kinetics of the conversion of prothrombin to thrombin are still perhaps a mysterious, but certainly a controversial question. If, however, we shift our interest to the bleeding patient, it becomes obvious that marked deficiency of any of the factors known to take part in the formation of thrombin, whether indispensable or not by in vitro studies, is accompanied by severe bleeding manifestations, an indication that the hemostatic mechanism has become grossly inadequate. From the clinician's point of view, effective protection against hemorrhage requires the presence in concentrations above the critical level of *all* factors known to play a role in the coagulation of blood.

The third phase of the clotting process consists in the *gelification of fibrinogen to fibrin.* A few authors believe that the reaction is proteolytic in nature. Laki[47] thinks that thrombin makes fibrin molecules from fibrinogen by splitting off peptides (these peptides contain approximately 3 per cent of the original N and are composed of at least twelve amino acids, including aspartic and glutamic acid). Lorand and Middlebrook[48] state that fibrinogen is converted to fibrin by losing two α-amino groups of glycine through the action of thrombin. These results, obtained with bovine fibrinogen, are probably not applicable to human physiology in their entirety. Still another point of view has been postulated, with Lyons[49] as its most vigorous proponent. It is believed that thrombin is able to oxidize the sulfhydril (SH-HS to S-S) group of fibrinogen. According to Lyons, the fibrinogen → fibrin reaction would occur in two steps: first, the S-H groups of fibrinogen would be freed;

secondly, the S-H groups would be oxidized, probably by the activity of the naphthoquinone groups present in the thrombin molecule. Fibrinogen B has been identified by Lyons as an intermediary product of the fibrinogen → fibrin conversion. Curiously enough, according to Lyons, fibrinogen B can be easily precipitated from plasma by the addition of β-naphtol in patients with thromboembolism or thrombotic tendency. Due to the obvious diagnostic importance of such findings, we and others have tried to confirm Lyons' results, but without success. The prevailing view today is that human fibrinogen and fibrin are essentially identical and exhibit identical antigenic properties. They yield identical degradation products on enzymatic digestion. The nature of the fibrinogen → fibrin reaction seems to consist of a molecular rearrangement. It appears that, after a preliminary end-to-end arrangement, the long molecules of fibrinogen undergo a process of tri-dimensional polymerization.[50, 51] Thus fibrin comes into being, the fibrinogen molecules not losing their individuality. The significance of other coagulation factors in the fibrinogen → fibrin reaction is not clearly defined. It is known that platelet extracts (platelet factor 2) accelerate the conversion of fibrinogen and that calcium may play an important role in the primary process of polymerization of the fibrinogen molecules.[51] The fate of thrombin itself is not very clear. It is possible that it might unite temporarily with fibrinogen while this is converted to fibrin. This fibrinogen-thrombin reversible combination may represent the less soluble, less stable and already partially denatured fibrinogen derivative (different from fibrinogen B), defined by Apitz as *profibrin.* Thus, even in this apparently clear-cut phase of the blood coagulation mechanism, a number of important questions remain unanswered.

At the end of this analytical and necessarily concise presentation of the various phases of the clotting mechanism as we visualize it in the test tube, it is perhaps useful to try to visualize the sequence of events as they might occur dynamically "in vivo" (Fig. 15). Platelet agglutination and lysis are the first step of the process. As platelet products become free, AHG is utilized (the role of PTC and PTA remaining to be better explored) and thromboplastin develops. Thromboplastin, calcium, labile and stable factors then form probably several intermediary complexes and prothrombin is converted to thrombin. As thrombin is formed, labile and, perhaps, stable factors are activated, thrombin formation is greatly accelerated and the fibrin clot appears. Such concept

FIGURE 15—DIAGRAMMATIC REPRESENTATION OF THE SEQUENCE OF EVENTS AND OF THE FATE OF VARIOUS CLOTTING FACTORS DURING THE PROCESS OF COAGULATION OF NATIVE PLASMA.*



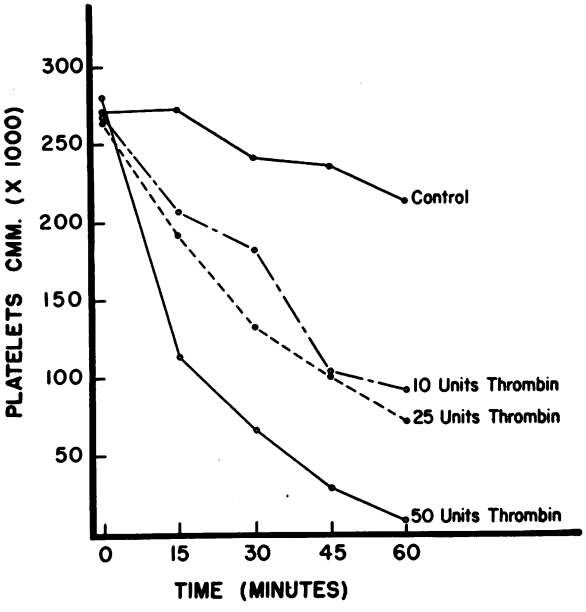
* Platelets are agglutinated and presumably lysed and, at the same time, antihemophilic globulin activity decreases rapidly and thromboplastin appears in the plasma. Then, as the activity of prothrombin and labile factor decreases rapidly, serum accelerator and thrombin are found in increasing concentration. As the activity of thrombin increases, fibrinogen is converted to fibrin. At a later time, but within few minutes of the completion of clotting, the activity of thrombin and serum accelerator decreases rapidly. PTC, stable factor and calcium are not included, since they do not seem to undergo any significant change of activity during clotting. Most of these data were obtained from the study of a patient with severe acquired (but selective) hypofibrinogenemia and agree fairly well with those described by Alexander et al. in cases of congenital afibrinogenemia. (Abstract, *Fed. Proc. 12*:167, 1953).

is, of course, highly hypothetical, but does not disagree radically with any of the observations in the test tube and those obtained in living animals by methods which visualize the circulation of blood in the capillary bed and the changes following hemorrhage.

THE AUTOCATALYTIC MECHANISMS

The final consideration of the preceding section leads us to discuss other basic mechanisms of hemostasis, those of autocatalysis. In man, the hemostatic process cannot be fully effective in the control of bleeding, unless a solidly anchored fibrin clot can be formed. This, in turn, requires sufficient accumulation of thrombin at the site of vascular injury, accumulation which is limited by the washing effect of blood pouring from the wound, while the enzyme is also neutralized by various inhibitory agents. The autocatalytic mechanism assures that enough thrombin and other important clotting factors are available at the site

FIGURE 16—EFFECT OF PURIFIED HUMAN THROMBIN ON THE COUNT OF SUSPENSIONS OF PLATELETS IN DEPROTHROMBINIZED SERUM.



* From Stefanini, M.,[52] in *Acta med. scand. 140*:290-306, 1951. Reprinted by permission.

of injury. In a previous article we have discussed the development of the all-important concept that blood clotting, once initiated, proceeds at ever increasing speed and presented experimental evidence for it.[52] Experiments of many authors since Arthus seem to indicate that thrombin itself, the end product of the "preparatory" or "latent" phase of the coagulation process is, in effect, responsible for the autocatalytic phase of the clotting process. It is possible, although not established, that other factors in plasma or serum may be necessary for the initiation of the autocatalytic reaction by thrombin.

The autocatalytic mechanism involves two basic reactions, both apparently set in motion when a small amount of thrombin is formed: (a) agglutination and lysis of platelets; (b) conversion of the "labile factor" to "serum accelerator." It is possible that thrombin may also (c) convert stable factor into a more active agent, as postulated by Alexander[53] and Owren[26] but denied by others and, as Quick et al.[54] have recently postulated, (d) activate AHG prior to its reaction with platelets.

What is the available evidence that platelets and thrombin are involved in one of the autocatalytic mechanisms? It is known that no autocatalytic reaction takes place when no platelets are available and when no thrombin can be formed (as in decalcified plasma). This is, of course, extremely indirect evidence. A perhaps more direct approach is that of DesForges and Bigelow[55] who have demonstrated that the conversion of prothrombin in platelet-poor plasma is greater when thrombin-treated platelets rather than untreated platelets are added. Evidence of a more direct nature is also available. Thus, when purified human thrombin is added to a suspension of platelets in serum (even if this is deprothrombinized) platelets agglutinate and are lysed (Fig. 16). Recently, de Robertis et al.[56] have observed by electron microscope changes occurring in platelets after treatment with thrombin. As shown in Figure 17, platelets, even when washed free of plasma or serum, are lysed and disintegrated by purified thrombin.

The second autocatalytic mechanism involves the conversion of the labile factor to serum accelerator. Ware and Seegers[57] first showed that, in the test tube, purified thrombin will activate plasma to serum Ac-globulin. These two factors are very likely identical with labile factor and serum accelerator respectively. We and others were able to demonstrate two significant facts: (a) There is a direct quantitative relationship between amount of prothrombin utilized during clotting (and thus, presumably, of thrombin produced), labile factor activity lost and activity of serum accelerator developed during the coagulation of blood; (b) serum accelerator activity develops if deprothrombinized plasma (which has lost prothrombin and stable factor but retains the greatest amount of labile factor) is treated with thrombin. Since we know that serum accelerator is a better accelerator of the formation of thrombin than labile factor, the development of serum accelerator results ultimately in greater and faster formation of thrombin. This step may be considered the dividing line between the "slow", "latent" phase and the accelerated "autocatalytic" phase of the coagulation of blood.

The significance of the autocatalytic mechanisms of blood coagulation may deserve comment. We have mentioned already that anticoagulants and the washing effect of bleeding neutralize and wash away, respectively, thrombin and prevent its accumulation in amounts sufficient to form a firm fibrin clot. More subtle reasons can also be offered why it can be expected that, without a mechanism of autocatalysis, the

FIGURE 17

THE AUTO-CATALYTIC MECHANISM OF BLOOD COAGULATION

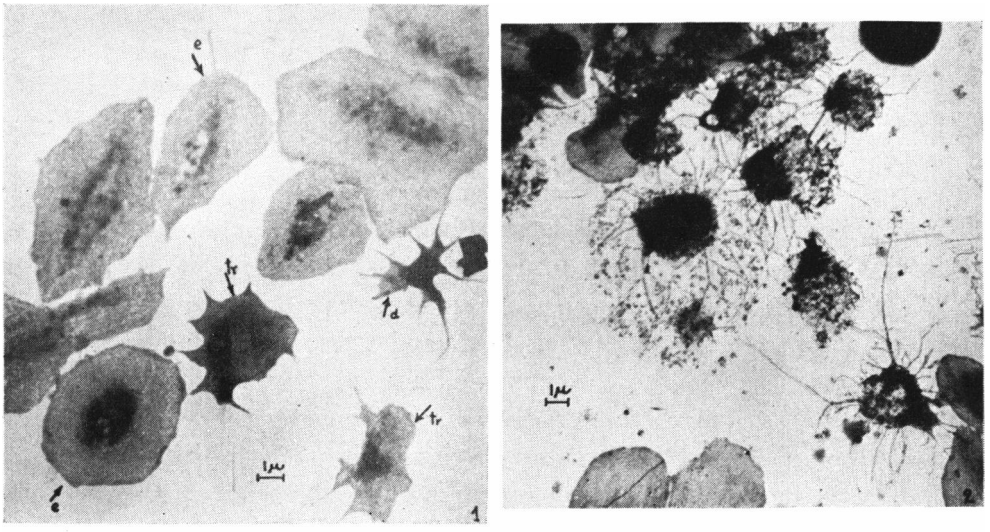
Thrombin may represent the key substance in this mechanism:

(1) It produces agglutination and lysis of platelets (possibly in the presence of a plasmatic factor) and, thus, the release of more platelet thromboplastic factor and platelet factors 1 and 2.

(2) It converts "labile factor" into "serum accelerator," thus greatly increasing the yield of thrombin and the speed of formation of this enzyme.

(3) It *may* also convert the "stable factor" into a more active derivative which is said to be present in serum.

THE EFFECT OF THROMBIN ON PLATELETS



17 a 17 b

THE AUTO-CATALYTIC MECHANISM REPRESENTS, HOWEVER, A POTENTIAL DANGER TO THE INDIVIDUAL

From DeRobertis, et al.[56] in *Blood* 8:587-97, 1953. Reprinted by permission of the authors and the publisher, Grune & Stratton, Inc.

formation of thrombin might be insufficient for hemostatic needs.[58] Without autocatalysis, any chemical reaction, stoichiometric or catalytic in nature, slows down after a period of maximum activity. Thus, the production of thrombin would tend to decrease as the process of blood coagulation continues. Also, the presence of an active autocatalytic agent (thrombin) on the surface of the clot guarantees that this will grow in size (as successive layers of fibrin are deposited) and will be anchored firmly. Finally, the continuous lysis of platelets maintains a steady supply of serotonin and this, in turn, assures steady vasoconstriction, necessary for a normal hemostatic mechanism.

The autocatalytic reaction may also help to explain why the defi-

FIGURE 18—PHYSIOLOGIC ANTICOAGULANTS

1. *All plasma proteins* (particularly globulins), capable of absorbing coagulation factors on their surfaces, thus decreasing their available concentration.
2. *Antithromboplastin*
*3. *Anti-accelerin* (?)
*4. *Anti-convertin* (?)
5. *Natural antithrombin*
6. *Heparin co-factor* (Albumin X)
7. *Fibrin clot*
8. POTENTIALLY: the fibrinolytic system (fibrinolysin and fibrinolysokinase), capable of destroying the formed fibrin clot.

* May represent an aspecific protein absorption effect.

ciency of single coagulation factors must be of severe degree before bleeding occurs. We see every day that the hemostatic mechanism is grossly normal and no bleeding occurs in patients with plasma prothrombin activity as low as 20 per cent, with labile factor activity as low as 25 per cent of normal, with platelet level of 60,000-70,000/c. mm. or less. The autocatalytic mechanisms possibly allow full utilization of the relatively deficient factors, and, thus, on it greatly depends the sufficiency of the hemostatic process in man.

ANTICOAGULANT MECHANISMS

The process of autocatalysis, while so important in maintaining efficient hemostasis and affording protection against hemorrhage, has in itself the impending danger of continued intravascular coagulation, until the damage caused to the organism through vascular occlusion might be greater than the benefit due to the prompt control of hemorrhage. Such ultimate possible effect of an otherwise extremely useful mechanism emphasizes the delicate balance upon which the hemostatic process is based.

The danger of extensive autocatalytic intravascular coagulation is checked and controlled by the availability of anticoagulant mechanisms (Fig. 18), some well defined, others which remain to be fully evaluated. *Absorption of active clotting agents on plasma proteins* (particularly globulins) as well as on fibrin may represent an aspecific but effective reaction. As clotting factors are absorbed onto the surface by proteins, their concentration is reduced and the clotting mechanism inhibited. It

is quite possible that hypothetical inhibitory agents against labile and stable factors (such as postulated by Owren) may be the expression of an absorption effect by plasma proteins. While this concept is mostly speculative, the antithrombin role of the *fibrin clot* has been well established by the studies of Foà[59] and Wilson.[60] The fibrin clot, large and spongious, is capable of absorbing thrombin on its surface, when this enzyme is produced at accelerated rate through the autocatalytic reaction. Fibrin clot removes formed thrombin from the site of injury thus decreasing the rate of the autocatalytic reaction, which depends on the concentration of available thrombin. The enzyme is, of course, slowly released later as the fibrin clot retracts, to be disposed of by the antithrombin mechanisms.

Very interesting from a theoretical standpoint is the presence of direct inhibitors of the first and second phase of the coagulation process (antithrombins and antithromboplastins) in the circulating blood. There is some evidence that at least two different *antithromboplastins* may be found in human plasma and serum. One, an inhibitor of *tissue* thromboplastin, is a water-soluble, heat labile factor requiring calcium for its activity.[61] The other, an inhibitor of *plasma* thromboplastin, is a lipoid substance extractable with ether or destroyed by it. It cannot be detected easily in normal plasma or serum, but it may be increased in rare bleeding conditions and, according to Tocantins, the hemostatic defect of hemophilia is, at least in part, due to its presence in excess. These two "physiologic" antithromboplastins should not be confused with another which may develop as the result of a process of iso-immunization in patients with hemophilia receiving multiple transfusions, in pregnant women with Rh-incompatible child, in pemphigus, lupus erythematosus, etc. This anticoagulant rarely inhibits thromboplastin directly,[62] most often inhibits the interaction of platelet and plasma factors to form thromboplastin.[63] Neutralization or inactivation of thrombin is the result of several mechanisms (Fig. 19). The thrombin-neutralizing role of the fibrin clot has been described already. Heparin, traces of which are apparently present in normal blood, is also able to neutralize thrombin if a plasmatic factor (albumin X) is also available. The most important thrombin neutralization mechanism, however, is probably represented by a heterogeneous group of factors found in the albumin fraction of plasma and serum and designated collectively as *antithrombin*. Thrombin is apparently absorbed onto the surface of antithrombin and this com-

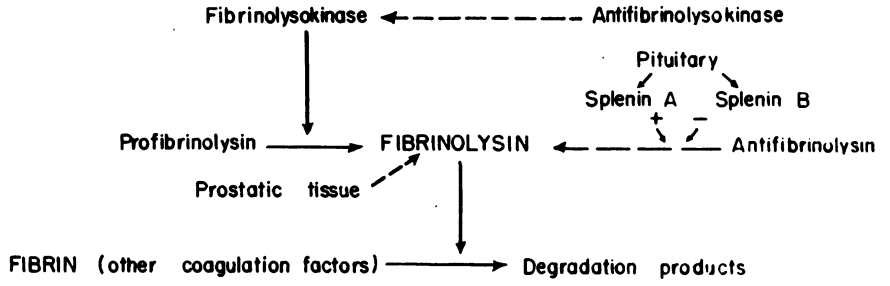
FIGURE 19—MECHANICS OF NEUTRALIZATION AND INACTIVATION OF THROMBIN

1. Interference of heparin and heparin co-factor (albumin X) with the action of thrombin on fibrinogen.
2. Neutralization of thrombin by natural antithrombin (the two agents form a reversible combination designated as METATHROMBIN).
3. Absorption of the thrombin on the fibrin clot.
4. Destruction of thrombin by a serum factor (?)

bination (which is reversible) is known as *metathrombin.* More recently, Seegers and associates[64] have postulated an additional antithrombin mechanism. At least in the dog, during and shortly after the coagulation and the activation of prothrombin, a substance develops in plasma from which antithrombin has been removed, capable of destroying thrombin activity. This substance apparently is one of the factors entering in the formation of thrombin.

In pathologic conditions, another antithrombic mechanism (in the broad sense of the term) can be found. Extremely rare cases of hemorrhagic tendency on the basis of deficient fibrin formation may be due to excess of individual plasma protein fractions. This occurs in "macroglobulinemia"[65] where hemorrhagic tendency is associated to an excess of "20S" ultracentrifugation plasma protein component, and, very occasionally, in cases of multiple myeloma when no significant thrombocytopenia is found but there is a striking increase of some of the globulin fractions. In both cases, the fibrinogen conversion by thrombin, perhaps the formation of thromboplastin are inhibited by the pathologic protein. The dilution of plasma in vitro and the replacement of abnormal with normal plasma in vivo correct the coagulation defect promptly but temporarily. High globulin concentration may also contribute to the bleeding tendency of liver dysfunction. As mentioned in the introductory remarks, these patients often present a very inclusive abnormality of the hemostatic mechanism. They occasionally have thrombocytopenia, very often reduction of activity and concentration of AHG, prothrombin, labile factor, stable factor, fibrinogen (due to liver insufficient synthesis of these various proteins). They also occasionally exhibit increased antithrombin activity, increased fibrinolytic activity and decreased vascular resistance (positive tourniquet test). In our experience, however, bleeding of patients with liver dysfunction is particularly im-

FIGURE 20—THE FIBRINOLYTIC SYSTEM IN HUMAN BLOOD.



Profibrinolysin (plasminogen, tryptogen, prolysin, lytic factor, etc.) found in plasma and serum is activated to *fibrinolysin* (plasmin, tryptase, tissue lysin, etc.) by a *fibrinolysokinase* (found in tissues, especially lung and uterus). The action of the kinase is probably inhibited or antagonized by inhibitors present in plasma and serum (*antifibrinolysokinases*). The active fibrinolysin is antagonized and inhibited by *antifibrinolysin* (antiplasmin), also found in plasma and serum. Alternatively, a proteolytic enzyme (similar to, but not identical with fibrinolysin) of prostatic origin may also lyse fibrin clots (disseminated prostatic carcinoma). Moreover, other proteolytic enzymes may also be present in human plasma as inert precursors and be occasionally activated by similar mechanisms.

pressive when there is considerable alteration of the plasma protein pattern, even when the coagulation defect is not very significant by laboratory tests. This suggests that the hyperglobulinemia of these patients may play a role in the pathogenesis of their bleeding tendency.

THE FIBRINOLYTIC MECHANISM (Figure 20)

There exists in the circulating plasma a pro-enzyme (profibrinolysin). The pro-enzyme is not capable of attacking any of the coagulation proteins. It may do so after being activated by bacterial filtrates (staphylokinase; streptokinase; etc.) or by tissue extracts (fibrinolysokinase) in vitro and in vivo its activation may be due to the liberation of tissue kinases (in which lung and uterine tissues are particularly rich), to the activity of bacteria, and finally, to liberation of adrenalin ("alarm reaction"). Plasma, and serum, however, contain two antagonistic agents: antifibrinolysokinase (which opposes the activation of fibrinolysin) and antifibrinolysin which quickly combines with, and neutralizes, fibrinolysin. Antifibrinolysin is able to combine with fibrinolysin, at least in vitro, according to definite quantitative proportions, the reaction being completed within sixty to ninety minutes. For this reason, and if no constant supply of active fibrinolysin is available, clot lysis is of short duration even when massive activation has taken place.

FIGURE 21—SIGNIFICANT FIBRINOLYSIS MAY BE FOUND IN THE FOLLOWING CONDITIONS

1. Hemorrhage, shock, trauma, "stress"
2. Extensive surgery (especially pulmonary, pancreatic, etc.)
3. Obstetrical accidents (abortion, premature separation of placenta(*), etc.)
4. Leukemic states
5. Parenchymal liver disease (especially cirrhosis)
6. Transfusion reactions (hemolytic, "plasma transfusion reaction")
7. Metastatic carcinoma of the prostate

* An alternative explanation postulates that thromboplastic substances of placental origin may pass into the maternal circulation and induce intravascular clotting: fibrinogen and other coagulation proteins would then be consumed and severe bleeding tendency develop. The two explanations may possibly be integrated, since activation of fibrinolysin may follow attempts to intravascular clotting, as in "plasma transfusion reaction."

Also, there is an endocrine control of the fibrinolysin-antifibrinolysin complex, which has been described in detail by G. Ungar and Damgaard.[66] In the guinea pig the spleen probably produces two opposing factors: *splenin A* which accelerates, and *splenin B* which decelerates the inactivation of fibrinolysin by antifibrinolysin. In man and in pathologic conditions of active fibrinolysis, ACTH and cortisone may quickly decrease the plasma fibrinolytic activity.[67] We have found these hormones of great therapeutic value in the control of fibrinolytic crises.

The careful control of the mechanism of activation of fibrinolysin and of the activity of the free enzyme is of extreme importance. This quickly becomes apparent when one considers the disastrous consequences of the activation of fibrinolysin, which is accompanied by very severe bleeding tendency. This is seen in a series of clinical conditions (Fig. 21). Thoracic and pancreatic surgery,[68] some of the cases of obstetrical accidents, such as premature separation of placenta, shock, severe hemorrhage, are followed by increased fibrinolytic activity. Marked activation of fibrinolysin follows an attempt at intravascular coagulation in hemolytic transfusion reaction and "plasma transfusion reaction." In leukemia and parenchymal liver disease, fibrinolysis is also present. There is some contention as to whether active fibrinolysis may destroy other coagulation proteins besides fibrin.[69] If streptokinase may be considered to activate only profibrinolysin, then our experiments with the intravenous injection of purified streptokinase- streptodornase preparations, clearly point out that this is so, although the possibility exists that kinases may activate not only profibrinolysin but precursors

of other proteolytic enzymes as well. Finally, a preformed fibrinolytic enzyme, similar to but not identical with fibrinolysin, enters the circulation in cases of disseminated prostatic carcinoma, as demonstrated by Tagnon et al.[70, 71] This enzyme destroys not only fibrin, but also fibrinogen, prothrombin and other accessory factors, inducing a most alarming hemorrhagic picture.

While fibrinolysis is a very important pathogenetic mechanism of hemorrhage in pathologic conditions, there is no definite evidence that the enzyme plays any role in the normal mechanism of blood coagulation. It has been postulated that fibrinolysin may be responsible for the initiation of blood clotting, but no clear-cut experimental evidence of this concept has been obtained. The limited activation of profibrinolysin which accompanies the coagulation of blood may be interpreted in favor of the concept that the enzyme cooperates in limiting the extension of the intravascular clot of fibrin. It is obvious, however, that our present knowledge is too limited to attempt an evaluation of the role of fibrinolysin in the normal hemostatic process.

Conclusion

Perhaps a few final remarks are in order in closing this presentation. In preparing it, I had meant to try and harmonize the many valuable contributions in the field, particularly for the past ten years, which have witnessed a veritable revival of interest and progress in the subject of hemostasis. This goal proved to be an even harder task than expected and, I rather suspect, an ungrateful one. Many unvoluntary omissions became unavoidable and I found it most difficult to prevent myself from expressing my own feelings and ideas more often than I have done. One's own work, I am afraid, generates often preconceived ideas, especially when one has behind him years of investigation, elating at times but more often frustrating, and of interpretative effort.

To prepare this presentation, however, has also been a refreshing and encouraging experience. While the multiplicity of hypotheses and the conflict of experimental findings still deny us a firm theoretical basis for the interpretation of the mechanisms of hemostasis, the impact of the advances of the last ten years on the diagnosis and management of the bleeding patient have been staggering. New diagnostic tests have greatly increased the accuracy of the diagnosis; broader interest in the isolation of coagulation factors and of platelets points to more specific methods

of treatment in the near future. One feels that, with the unending ferment of ideas and fervor of investigation in this field, great progress lies ahead.

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